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# LIF and the lung's stem cell niche: is failure to use LIF to protect against COVID-19 a grave omission in managing the pandemic?

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“leukemia inhibitory factor (LIF) – a key stem cell growth factor – is required to maintain continued function of the blood–air barrier during infection, identifying LIF as a vital factor in the current global battle against COVID.”

**Tweetable abstract:** What tips the SARS/COVID-19 balance into severe pneumonia, rather than recovery? Is it insufficient LIF – the lung's own protective growth factor at the blood–air barrier?

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## What tips the balance into COVID-19 pneumonia?

Pulmonary infections represent the world's greatest burden of disease, reflecting the vulnerability of the lung's blood–air barrier to pathogens. Despite intense efforts to bring the current COVID-19 pandemic under control, the highly virulent SARS-CoV-2 continues to spread from human to human with insidious ease and devastating effect. Case fatality rate reaches 13% with Italy currently at 11% [1]. Survivors become spreaders due to prolonged shedding of virus, while symptoms in those newly infected are preceded by several days shedding, further exacerbating spread.

The speed of transition from SARS-CoV-2 infection into COVID-19 pneumonia is alarming, evidenced in a cynomolgus macaque model of human asymptomatic disease [2].

Day 0: Infection: Intratracheal and intranasal SARS-CoV-2 (total dose  $10^6$  TC LD<sub>50</sub>);

Day 4: Acute pathogenic outcomes include:

- some 10% loss of lung volume with large foci of consolidation;
- foci show alveolar air sacs filled with inflammatory exudate
- Presence of viral nucleocapsid in alveolar type I and type II pneumocytes

Day 7: Sera-negative for viral-specific antibody;

Day 14: Sera-positive for viral antigens, mainly against viral spike protein. Implies at the time of a positive antibody test, infection is over 7 days old.

In people, three outcomes following SARS-CoV-2 infection have become apparent:

1. Full recovery;
2. 'Long COVID': Although some 80% of people infected by SARS-CoV-2 remain well or have mild, transient symptoms, some 10% who recover develop long COVID – a legacy of vascular and organ dysfunction involving not only the lung but also variably heart, liver, kidney and brain, accompanied by profound fatigue. The asymptomatic macaque study [2] alerts to the rapid speed and potential for silent spread of disease that might underlie delayed manifestations of long COVID in some patients;

3. Acute COVID: Here 20% of those infected rapidly progress to pneumonia. Although unpredictable who will succumb, risk factors show a correlation with elderly age, raised circulating IL-6 (inflammatory driver of cytokine storm) and with comorbidities independent of age [3].

Questions arise:

- What is the tipping point? What is happening in the lung, at around day 4–7, that tips the balance into worsening acute disease rather than recovery?
- Can the risk factors acting at the tipping point be modified?
- Can the risk of long COVID be avoided?

This brief editorial develops a case for the tipping point being integral to the lung's own stem cell niche. Here leukemia inhibitory factor (LIF) – a key stem cell growth factor – is required to maintain continued function of the blood–air barrier during infection, identifying LIF as a vital factor in the current global battle against COVID.

### Natural resistance to infection at the blood–air barrier

We know COVID pneumonia is precipitated by death of the lung's delicate alveolar cells that create the lung's blood–air barrier. Alveoli are the air sacs of the lung where some 700 million alveoli amount to a surface area of 70 m<sup>2</sup>, representing a vast expanse of vulnerable cells for gaseous exchange to deliver oxygen to the blood. The alveolar wall is only one cell thick. It backs onto the vascular endothelial cells of the alveoli's blood capillaries – also only one cell thick – allowing unimpeded gaseous exchange between air and erythrocytes across the two-cell thick blood–air barrier.

Having evolved a respiratory system dependent on oxygen, the mammalian lung has co-evolved defense mechanisms to protect its alveoli. LIF is a vital multifunctional stem cell growth factor acting in cooperation with pioneer transcription factors to provide genetic plasticity sensitive to micro-environmental cues [4]. LIF's property of 'stemness' operates throughout life supporting adult stem cells and precursor cells, including the stem cell niche of the lung's blood–air barrier. Notably, in human, lack of LIF signaling results in 'Stuve–Wiedemann syndrome', a rare genetic disorder where infants may succumb to respiratory distress by around 3 months of age [5].

In animal models of pneumonia LIF was found to protect against severe disease, since removal of endogenous LIF by anti-LIF antibody resulted in acute respiratory distress syndrome often accompanied by sepsis: this was despite all other growth factors and cytokines being present – identifying LIF as unique and essential in protecting lung function [6,7]. Another study showed recombinant therapeutic LIF, delivered either intratracheal or intravenous, prevented acute respiratory distress syndrome [8].

LIF's protective role includes being anti-inflammatory for vascular endothelial cells [9], preserving integrity of the vessel lining, so preventing leak during inflammation as evident in the pneumonia model, where specific removal of LIF resulted in excessive vascular leak into the alveoli [6]. Cytokine storm is accompanied by vascular leak in COVID-19, linked to acute vasculitis and abnormal clotting [10]. Since LIF both protects against cytokine storm (see later) and reduces vascular endothelial damage, a place for intravenous therapeutic LIF to treat severe COVID-19 warrants consideration.

### The mechanism of the tipping point

The alveolus consists of only two cell types creating its single cell thick air sac wall. Termed type I and type II pneumocytes, the type I cells provide most of the wall's surface area, while the type II cells perform specific functions: first, releasing a fatty 'surfactant' that lines the alveolar air space, keeping it elastic during breathing in and out. Without surfactant the air sac collapses. Second, type II cells operate as the lung's own stem cell niche [11], providing a reservoir of new-born type I and type II cells to replace any that die.

Now the reason why SARS-CoV-2 virus infection can be devastating becomes clear: the type II cells are in line for first attack by inhaled virus because they express angiotensin-converting enzyme 2, the docking site for the virus to enter the lung [12]. Viral infection of alveolar type II cells was evident at day 4 in the monkey coinciding with foci of alveoli filled with fluid, inflammatory cells and debris. This is a 'tipping point'. If too many alveolar type II cells die, the collapse of air sacs reaches a point where the lung can no longer breathe and the alveoli lose the ability to repair themselves because they have lost their stem cell niche.

### What is the trigger for endogenous LIF to be released during infection?

LIF is not normally evident in the mature lung – only when infected. So, how does the lung coordinate LIF activity to infection? The evidence supports a story where a physiological ‘twist’ operates: alveolar macrophages are specialized cells that crawl around the alveolar surface, removing any inert debris. In addition to keeping the alveolus clean, they also act as guard cells, patrolling the blood–air barrier as first line of defense against any invading virus. When virus is encountered the macrophages release an alarm signal in the form of inflammatory cytokines, activating the innate and adaptive immune systems to mount inflammatory attack against the virus. But – remarkably – the same inflammatory cytokine alarm also triggers production of LIF in the alveoli: within hours LIF gene expression is upregulated, followed by high levels of LIF protein release [7,13,14] – protecting the type I and type II cells from collateral damage during inflammatory attack against the virus. If this LIF is removed – the alveoli die [6]. But with LIF, not only are the vulnerable cells protected but also any damage is healed and the type II cells – when in sufficient numbers – prevent scarring and fibrosis within the recovered lung [15].

### Can the risk factors acting at the tipping point be modified? Can the risk of long COVID be avoided?

As fore mentioned, severity of COVID-19 correlates with both age and IL-6 [3]. The clue, linking age to IL-6, is important, since IL-6 generally increases with age, while LIF generally decreases. A hierarchical LIF/IL-6 axis cross-regulates tolerant versus inflammatory immunity [16]. Thus, the age-related skew against LIF will favor escalating IL-6 inflammatory activity because levels of LIF will no longer be sufficient to downregulate the IL-6 pathway. This is a second, age-linked tipping point. Importantly, evidence shows the tilted LIF/IL-6 axis can be reset to equilibrium by exogenous delivery of therapeutic LIF, either soluble or as targeted high potency nanoparticles [16,17], with potential to repress inflammation and favor vascular recovery.

Overall, the lung’s own physiology argues a case for inclusion of LIF as part of the current armamentarium in management of the COVID-19 pandemic. When safety of repurposed therapeutic LIF is confirmed, the potential of a resource able to directly protect the lung from viral pathogenesis will provide for:

- High-risk pre-exposure prophylaxis;
- High-risk postexposure prophylaxis;
- Mild disease treatment (outpatient);
- Moderate disease treatment (early hospitalization).

Previous Phase I and II clinical trials showed safety of recombinant human LIF at 4 µg/kg subcutaneously daily for 7 days [18] while in practice bulk GMP manufacture together with regulatory approval for a trial in pneumonia is feasible within 12 months. With initial calculation of cost per dose being some £200, this contrasts to current clinical use of cell-based therapies for COVID [19] – an indirect source of growth factors including LIF [20].

Building on the evidence above, I propose that recombinant human LIF is a realistic treatment option to directly protect the lung from COVID-19 – being cell-free and off-the shelf, universal rather than viral specific, and complementary to vaccines and drugs.

#### Author contributions

SM Metcalfe is sole author and contributor.

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